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14 November 2003

VIA EXPRESS MAIL

PCT International Application Processing Div.
USPTO International Division
Assistant Commissioner for Patents
Mail Stop PCT
P.O. Box 1450
Alexandria, VA 22313-1450

Re: International Application No. PCT/US03/11867
Title: METHODS OF ASSAYING FOR CELL CYCLE MODULATORS
Applicant: RIGEL PHARMACEUTICALS, INC.
Filed: 15 April 2003
Express Mail Label No.: EV 332 020 720 US
Date of Mailing: 14 November 2003
Our File No.: 21044-33-1PC

Dear Examiner:

Enclosed are the Chapter II Demand and nine (9) substitute Specification pages 6, 7, 8, 8a, 9, 9a, 14, 61, and 77 submitted as an Article 34 Amendment. The only changes were insertions of SEQ ID:NOs. and corrections of typographical errors that do not include matter which goes beyond the disclosure in the international application as filed.

It is hereby stated that "the information recorded on the computer readable form is identical to the written sequence listing" and does not include matter which goes beyond the disclosure in the international application as filed.

Thank you for your attention in this matter.

Respectfully submitted,

TOWNSEND and TOWNSEND and CREW LLP

Beth L. Kelly
Reg. No. 51,868

Enclosures:
Chapter II Demand
Article 34 Amendment
Nine (9) Substitute Specification pages 6, 7, 8, 8a, 9, 9a, 14, 61, and 77
Fifty-four (54) pages of Sequence Listing
Diskette & Statement
Transmittal Letter
Postcard

60079957v1

The demand must be filed directly with the competent International Preliminary Examining Authority; if two or more Authorities are competent, with the one chosen by the applicant; the full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ US

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION

Applicant's or agent's file reference: 21044-33-1PC

International application No.	International filing date (*day/month/year*)	(Earliest) Priority date (*day/month/year*)
PCT/US03/11867	15 April 2003 (15.04.2003)	15 April 2002 (15.04.2002)

Title of invention

METHODS OF ASSAYING FOR CELL CYCLE MODULATORS

Box No. II APPLICANT(S)

Name and address: (*Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.*)

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1180 Veterans Blvd.
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Telephone No.: 650-624-1100
Facsimile No.: 650-624-1101
Teleprinter No.:
Applicant's registration No. with the Office

State (*that is, country*) of nationality:	State (*that is, country*) of residence:
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JENKINS, Yonchu
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State (*that is, country*) of nationality:	State (*that is, country*) of residence:
US	US

☐ Further applicants are indicated on a continuation sheet.

International application No.
PCT/US03/11867

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is ☒ agent ☐ common representative

and ☒ has been appointed earlier and represents the applicant(s) also for international preliminary examination.

☐ is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: (*Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.*)

KELLY, Beth, L.
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Agent's registration No. with the Office: 51,868

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination **to start on the basis of:**

☒ the international application as originally filed

the description
- ☐ as originally filed
- ☒ as amended under Article 34

the claims
- ☒ as originally filed
- ☐ as amended under Article 19 (together with any accompanying statement)
- ☐ as amended under Article 34

the drawings
- ☒ as originally filed
- ☐ as amended under Article 34

2. ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. ☐ The applicant wishes the start of the international preliminary examination **to be postponed** until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). (*This check-box may be marked only where the time limit under Article 19 has not yet expired.*)

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: ENGLISH

☒ which is the language in which the international application was filed.

☐ which is the language of a translation furnished for the purposes of international search.

☐ which is the language of publication of the international application.

☐ which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby **elects all eligible States** (*that is, all States which have been designated and which are bound by Chapter II of the PCT*)

excluding the following States which the applicant wishes **not to elect:**

International application No.
PCT/US03/11867

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

				For International Preliminary Examining Authority use only: received	not received
1.	translation of international application	:	sheets	☐	☐
2.	amendments under Article 34	: 9	sheets	☐	☐
3.	copy (or, where required, translation) of amendments under Article 19	:	sheets	☐	☐
4.	copy (or, where required, translation) of statement under Article 19	:	sheets	☐	☐
5.	letter	: 1	sheets	☐	☐
6.	other (*specify*)	:	sheets	☐	☐

The demand is also accompanied by the item (s) marked below:

1. ☒ fee calculation sheet
2. ☐ original separate signed power of attorney
3. ☐ original general power of attorney;
4. ☐ copy of general power of attorney; reference number, if any:
5. ☐ statement explaining lack of signature
6. ☒ sequence listing in computer readable form
7. ☐ tables in computer readable form related to sequence listings
8. ☒ other (*specify*) Transmittal Letter, Postcard, Sequence Listing Statement Letter & Diskette

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

X _____

Beth L. Kelly
TOWNSEND AND TOWNSEND AND CREW LLP
USPTO Reg. No.: 51,868
Applicants' Agent

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. ☐ The applicant has been informed accordingly.
4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

CHAPTER II

PCT

FEE CALCULATION SHEET

Annex to the Demand

For International Preliminary Examining Authority use only

International application No.	PCT/US03/11867
Applicant's or agent's file reference	21044-33-1PC

Date stamp of the IPEA

Applicant

RIGEL PHARMACEUTICALS, INC.

CALCULATION OF PRESCRIBED FEES

1. Preliminary examination fee	$490.00	P
2. Handling fee (*Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.*)	$172.00	H
3. Total of prescribed fees Add the amounts entered at P and H and enter total in the TOTAL box	$662.00	TOTAL

MODE OF PAYMENT

- ☒ authorization to charge deposit account with the IPEA (see below)
- ☐ cash
- ☐ cheque
- ☐ revenue stamps
- ☐ postal money order
- ☐ coupons
- ☐ bank draft
- ☐ other (*specify*):

AUTHORIZATION TO CHARGE (OR CREDIT) DEPOSIT ACCOUNT

(*This mode of payment may not be available at all IPEAs*)

The IPEA/ US

☒ is hereby authorized to charge the total fees indicated above to my deposit account.

☒ (*this check-box may be marked only if the conditions for deposit accounts of the IPEA so permit*) is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

20-1430	14 November 2003	Beth L. Kelly
Deposit Account Number	Date (*day/month/year*)	Signature

threonine kinase 15 (ARK2), transmembrane 4 superfamily member 1, or ERCC1 polypeptide may be encoded by a nucleic acid that hybridizes under stringent conditions to a nucleic acid encoding a polypeptide having an amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, or 28.

A further embodiment of the invention provides a method of modulating cell cycle arrest in a subject. A therapeutically effective amount of a BRCA-1-Associated Protein-1 (BAP-1), Nuclear Protein 95 (NP95), Fanconi anemia group A protein (FANCA), DEAD/H box polypeptide 9 (DDX9), insulin-like growth factor 1 receptor (IGF1R), ubiquitin-conjugating enzyme E2 variant 1 (UBE2V1), aldehyde dehydrogenase, pyruvate kinase, glucose-6-phosphate dehydrogenase, HCDR-3, DEAD/H box polypeptide 21 (DDX21), serine threonine kinase 15 (ARK2), transmembrane 4 superfamily member 1, or ERCC1 polypeptide is administered to the subject. The BRCA-1-Associated Protein-1 (BAP-1), Nuclear Protein 95 (NP95), Fanconi anemia group A protein (FANCA), DEAD/H box polypeptide 9 (DDX9), insulin-like growth factor 1 receptor (IGF1R), ubiquitin-conjugating enzyme E2 variant 1 (UBE2V1), aldehyde dehydrogenase, pyruvate kinase, glucose-6-phosphate dehydrogenase, HCDR-3, DEAD/H box polypeptide 21 (DDX21), serine threonine kinase 15 (ARK2), transmembrane 4 superfamily member 1, or ERCC1 polypeptide may be encoded by a nucleic acid that hybridizes under stringent conditions to a nucleic acid encoding a polypeptide having an amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, or 28.

Other embodiments and advantages of the present invention will be apparent from the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides a nucleotide (SEQ ID NO:1) and amino acid sequence (SEQ ID NO:2) of human BAP-1.

Figure 2 provides an illustration of the relevant domains of BAP-1, including the ubiquitin hydrolase domain and the DNA binding domain. Also shown is the BAP-1 functional hit (G3-2D8; SEQ ID NOS:36 and 37) isolated in the retroviral screen. The functional hit is in the antisense orientation BstXI linkers = SEQ ID NOS:38 and 39.

Figure 3 illustrates cell tracker assay data demonstrating that GFP-fused BAP-1 is antiproliferative in A549 cells. The BAP-1 construct is the functional hit isolated in the retroviral screen. Figure 3 top left illustrates fluorescence analysis of green fluorescent protein (GFP) infected A549.tTA control cells. Figure 3 top right illustrates cell tracker assay

data from GFP infected A549.tTA control cells. Figure 3 lower left illustrates fluorescence analysis of BAP-1 infected A549.tTA cells. Figure 3 lower right illustrates cell tracker assay date from BAP-1 infected A549.tTA cells.

Figure 4 provides a nucleotide (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of human NP95.

Figure 5 provides an illustration of the relevant domains of NP95, including the ubiquitin like domain, the zinc finger domain, the nuclear protein domain, and the ubiquitin ligase domain G1-2635 = SEQ ID NO:40.

Figure 6 illustrates cell tracker assay data demonstrating that GFP-fused NP95 is antiproliferative in A549. The NP-95 construct is the functional hit isolated in the retroviral screen. Figure 6 top left illustrates fluorescence analysis of green fluorescent protein (GFP) infected A549.tTA control cells. Figure 6 top right illustrates cell tracker assay data from GFP infected A549.tTA control cells. Figure 6 lower left illustrates fluorescence analysis of NP95 infected A549.tTA cells. Figure 6 lower right illustrates cell tracker assay date from NP95 infected A549.tTA cells.

Figure 7 provides a nucleotide (SEQ ID NO:5) and amino acid (SEQ ID NO:6) sequence of human FANCA.

Figure 8 provides a nucleotide (SEQ ID NO:7) and an amino acid (SEQ ID NO:8) sequence of human DDX9. DEAD (Asp-Glu-Ala-Asp) box motif = SEQ ID NO:60.

Figure 9 provides a nucleotide (SEQ ID NO:9) and an amino acid (SEQ ID NO:10) sequence of human IGF1R.

Figure 10 provides a nucleotide (SEQ ID NO:11) and an amino acid (SEQ ID NO:12) sequence of human UBE2V1.

Figure 11 provides a nucleotide (SEQ ID NO:13) and an amino acid (SEQ ID NO:14) sequence of human aldehyde dehydrogenase.

Figure 12 provides a nucleotide (SEQ ID NO:15) and an amino acid (SEQ ID NO:16) sequence of human pyruvate kinase.

Figure 13 provides a nucleotide (SEQ ID NO:17) and an amino acid (SEQ ID NO:18) sequence of human G6PD.

Figure 14 provides a nucleotide (SEQ ID NO:19) and an amino acid (SEQ ID NO:20) sequence of human HCDR-3.

Figure 15 provides a nucleotide (SEQ ID NO:21) and an amino acid (SEQ ID NO:22) sequence of human DDX21.

Figure 16 provides a nucleotide (SEQ ID NO:23) and an amino acid (SEQ ID NO:24) sequence of human ARK2.

Figure 17 provides a nucleotide (SEQ ID NO:25) and an amino acid (SEQ ID NO:26) sequence of human transmembrane 4 superfamily member 1.

Figure 18 provides a nucleotide (SEQ ID NO:27) and an amino acid (SEQ ID NO:28) sequence of human ERCC1.

Figure 19 provides an illustration of certain relevant domains of FANCA, including the aldehyde dehydrogenase cysteine active site, FKBP-type peptidyl-prolyl cis-trans isomerase signature 1 site, the PX site, and the peptidase S8 site. G2-2F3 = SEQ ID NO:41.

Figure 20 illustrates cell tracker assay data demonstrating that GFP-fused FANCA is antiproliferative in A549 cancer cells. The FANCA construct is the functional hit isolated in the retroviral screen. Figure 20 top left illustrates fluorescence analysis of green fluorescent protein (GFP) infected A549.tTA control cells. Figure 20 top right illustrates cell tracker assay data from GFP infected A549.tTA control cells. Figure 20 lower left illustrates fluorescence analysis of FANCA infected A549.tTA cells. Figure 20 lower right illustrates cell tracker assay date from FANCA infected A549.tTA cells.

Figure 21 provides an illustration of certain relevant domains of DDX9, including the double stranded RNA binding motif, the DEAD/H box helicase domain, the helicase conserved C terminal domain, and the CLN3 protein domain. G3-2H6 = SEQ ID NOS:42 and 43; BstXI linkers = SEQ ID NOS:38 and 39; DEAD box = SEQ ID NO:60.

Figure 22 illustrates cell tracker assay data demonstrating that GFP-fused DDX9 is antiproliferative in A549 cancer cells. The DDX9 construct is the functional hit isolated in the retroviral screen. Figure 22 top left illustrates fluorescence analysis of green fluorescent protein (GFP) infected A549.tTA control cells. Figure 22 top right illustrates cell tracker assay data from GFP infected A549.tTA control cells. Figure 22 lower left illustrates fluorescence analysis of DDX9 infected A549.tTA cells. Figure 22 lower right illustrates cell tracker assay date from DDX9 infected A549.tTA cells.

Figure 23 provides an illustration of certain relevant domains of IGF1R, including the receptor L domain, the furin-like cysteine rich region, the fibronectin type II domain, the transmembrane domain, and the kinase domain. G3-2H2_1 = SEQ ID NO:44.

Figure 24 illustrates cell tracker assay data demonstrating that GFP-fused IGF1R is antiproliferative in A549. The IGF1R construct is the functional hit isolated in the retroviral screen. Figure 24 top left illustrates fluorescence analysis of green fluorescent

protein (GFP) infected A549.tTA control cells. Figure 24 top right illustrates cell tracker assay data from GFP infected A549.tTA control cells. Figure 24lower left illustrates

fluorescence analysis of IGF1R infected A549.tTA cells. Figure 24 lower right illustrates cell tracker assay date from IGF1R infected A549.tTA cells.

Figure 25 provides an illustration of the relevant domains of UBE2V1, including the ubiquitin conjugating enzyme domain. G3-2G2/2H2 = SEQ ID NOS:45 and 46; BstXI linkers = SEQ ID NOS:38 and 39.

Figure 26 illustrates cell tracker assay data demonstrating that GFP-fused UBE2V1 is antiproliferative in A549 cancer cells. The UBE2V1 construct is the functional hit isolated in the retroviral screen. Figure 26 top left illustrates fluorescence analysis of green fluorescent protein (GFP) infected A549.tTA control cells. Figure 26 top right illustrates cell tracker assay data from GFP infected A549.tTA control cells. Figure 26 lower left illustrates fluorescence analysis of UBE2V1 infected A549.tTA cells. Figure 26 top right illustrates cell tracker assay date from UBE2V1 infected A549.tTA cells.

Figure 27 shows portions of G3_2H2 (SEQ ID NOS:47 and 54) and portions of four alternatively spliced UBE2V1 transcripts (SEQ ID NOS:48-53, 55 and 56).

Figure 28 provides some cDNA sequence isolated from a cell tracker assay for cDNAs that regulate the cell cycle, *i.e.,* functional hits from the retroviral screen (SEQ ID NOS:29-35).

Figure 29 provides Uch-l3 (SEQ ID NO:57) and dominant negative mutants of BAP-1 (SEQ ID NO:58). Mutated residues are shown with arrows.

Figure 30 provides evidence that expression of Bap1 WT and protease mutants is antiproliferative in HeLa cells.

Figure 31 provides evidence tthat expression of Bap1 WT protein is antiproliferative in HeLa cells in the Celltracker assay.

Figure 32 provides evidence that expression of Bap1 protease mutants is slightly more antiproliferative than expression of Bap1 WT in H1299 cells.

Figure 33 provides evidence expression of Bap1 WT and Bap1 protease mutants is antiproliferative in H1299 cells in the Celltracker assay.

Figure 34 provides evidence that the Bap1 functional hit G32D8 is antiproliferative in HMEC cells.

Figure 35 provides evidence that the Bap1 functional hit G3-2D8 is antiproliferative in PrEC cells.

Figure 36 provides evidence that BAP1 specific siRNA has an antiproliferative effect on HeLa cells.

Figure 37 provides evidence that BAP1 specific siRNA induces G1 arrest in H1299 cells.

Figure 38 provides evidence that soluble GST-Bap1 protein can be expressed from SF9 cells. GST-Bap1 was produced using the baculovirus transfer vector pDEST20

Imamura, *et al., Nuc. Acids Res.* 26(9):2063 (1998); and Zhang *et al., J. Cell. Sci.* 112:2693 (1999)). Vectors containing DNA encoding DDX9 complement yeast that have mutations in *prp8-1*, the yeast homolog of DDX9 (*see* Imamura *et al.*). Helicase assays known to those of skill in the art can be used, e.g., to identify modulators of DDX9.

IGF1R encodes a cell surface tyrosine kinase receptor and binds to IGF1 ligand (*see, e.g.,* Nakae *et al., Endocr. Rev.* 22(6):818 (2001); Flier *et al., Proc. Nat'l Acad. Sci. USA* 83:664-668 (1086); Francke *et al., Cold Spring Harb. Symp. Quant. Biol.* 51(Pt. 2):855-866 (1986); Ullrich *et al., EMBO J.* 5:2503-2512 (1986); Cooke *et al., Biochem. Biophys. Res. Commun.* 177:1113-1120 (1991); Abbott *et al., J. Biol. Chem.* 267:10759-10763 (1992); Werner *et al., Proc. Nat'l Acad. Sci. USA* 93:318-8323 (1996); Grant *et al., J. Clin. Endocrinol. Metab.* 83:3252-3257 (1998); and Butler & LeRoith, *Endocrinology* 142(5):1685 (2001)). Upon ligand binding, the receptor undergoes a conformational change which enables it to bind ATP, thereby increasing their kinase activity and modulate cell proliferation (*see* Nakae *et al.*). IGF1R deficient mice develop cell proliferation disorders, including muscle hypoplasia due to decreased cell numbers; IGF1R null mice develop cell proliferation disorders including dwarfism (*Id.*). Overexpression of IGF1R has been linked to increased radioresistance of breast cancer cells (*see* Macaulay *et al., Oncogene* 22(6):4029 (2001)). Ligand binding assays, autophosphorylation assays, kinase assays, and signal transduction assays known to those of skill in the art can be used, e.g., to identify modulators of IGF1R.

UBE2V1 encodes a protein that has been show to play a role in cell cycle regulation (*see, e.g.,* Rothofsky *et al., Gene* 195:141-149 (1997); Sancho *et al., Mol. Cell. Biol.* 18:576-589 (1998); Ma *et al., Oncogene* 17:1321-1326 (1998); Hofmann & Pickart, *Cell* 96:645-653 (1999); Deng *et al., Cell* 103:351-361 (2000); and Thomson *et al., Genome Res.* 10:1743-1756 (2000)). Constitutive expression of exogenous UBE2V1 inhibits the capacity of colorectal adenocarcinoma cells to differentiate upon confluence and inhibits the mitotic kinase cdk1, thereby inducing the cells to arrest at the G_2-M phase of the cell cycle (*see,* Sancho *et al., Mol. Cell. Biol.* 18(1):576 (1998) and Stubbs *et al., Am. J. Path.* 154(5):1335 (1999)). UBE2V1 has four alternatively spliced transcripts that encode proteins with the conserved Ubc domain of E2 enzymes and unique N-terminal sequence (see Figure 27). Ubiquitination assays, e.g., ubiquitin ligase assays, known to those of skill in the art, can be used to identify modulators of UBE2V1.

Aldehyde dehydrogenases form a superfamily of NADP+ dependent enzymes that are involved in several distinct metabolic pathways (*see* Vasilou *et al., Chem. Biol.*

Synthetic polymers, such as polyurethanes, polyesters, polycarbonates, polyureas, polyamides, polyethyleneimines, polyarylene sulfides, polysiloxanes, polyimides, and polyacetates can also form an appropriate tag or tag binder. Many other tag/tag binder pairs are also useful in assay systems described herein, as would be apparent to one of skill upon review of this disclosure.

Common linkers such as peptides, polyethers, and the like can also serve as tags, and include polypeptide sequences, such as poly Gly sequences of between about 5 and 200 amino acids (SEQ ID NO:59). Such flexible linkers are known to persons of skill in the art. For example, poly(ethylene glycol) linkers are available from Shearwater Polymers, Inc. Huntsville, Alabama. These linkers optionally have amide linkages, sulfhydryl linkages, or heterofunctional linkages.

Tag binders are fixed to solid substrates using any of a variety of methods currently available. Solid substrates are commonly derivatized or functionalized by exposing all or a portion of the substrate to a chemical reagent which fixes a chemical group to the surface which is reactive with a portion of the tag binder. For example, groups which are suitable for attachment to a longer chain portion would include amines, hydroxyl, thiol, and carboxyl groups. Aminoalkylsilanes and hydroxyalkylsilanes can be used to functionalize a variety of surfaces, such as glass surfaces. The construction of such solid phase biopolymer arrays is well described in the literature. *See, e.g.*, Merrifield, *J. Am. Chem. Soc.* 85:2149-2154 (1963) (describing solid phase synthesis of, e.g., peptides); Geysen *et al., J. Immun. Meth.* 102:259-274 (1987) (describing synthesis of solid phase components on pins); Frank & Doring, *Tetrahedron* 44:60316040 (1988) (describing synthesis of various peptide sequences on cellulose disks); Fodor *et al., Science*, 251:767-777 (1991); Sheldon *et al., Clinical Chemistry* 39(4):718-719 (1993); and Kozal *et al., Nature Medicine* 2(7):753759 (1996) (all describing arrays of biopolymers fixed to solid substrates). Non-chemical approaches for fixing tag binders to substrates include other common methods, such as heat, cross-linking by UV radiation, and the like.

IMMUNOLOGICAL DETECTION OF BAP-1, NP95, FANCA, DDX9, IGF1R, UBE2V1, ALDEHYDE DEHYDROGENASE, PYRUVATE KINASE, G6PD, HCDR-3, DDX21, ARK2, TRANSMEMBRANE 4 SUPERFAMILY MEMBER 1, OR ERCC1 POLYPEPTIDES

In addition to the detection of BAP-1, NP95, FANCA, DDX9, IGF1R, UBE2V1, aldehyde dehydrogenase, pyruvate kinase, G6PD, HCDR-3, DDX21, ARK2,

Example 12: BAP-1 WT protein, protease mutants, siRNA and antisense functional hit are antiproliferative.

The BAP-1 functional hit identified in the retroviral screen is in the antisense orientation. (Figure 2). Expression of the functional hit in a tumor cell line, *e.g.*, A549 cells, or in untransformed cells, *e.g.*, HMEC or PrEc cells, was antiproliferative. (See, *e.g.*, Figures 3, and 34-35.)

Dominant negative mutants of BAP-1 were made by mutating residues in the protease domain. (See, *e.g.*, Figure 29.) Using two different assays, expression of BAP-1 wild-type and protease mutants was antiproliferative in tumor cell lines, *i.e.*, HeLa cells and H1299 cells. (See, *e.g.*, Figures 30-33). siRNA molecules derived from the BAP-1 nucleic aicd were shown to be antiproiferative in HeLa cells and H1299 cells. (See, *e.g.*, Figures 36-37.)

Example 13: BAP-1 is a ubiquitin protease.

GST-Bap-1 was expressed in and purified from SF9 cells. (See, *e.g.*, Figures 38-39.) Using a fluorogenic ubiuquiting cleavage assay, BAP-1 was shown to be an active ubiquitin protease, with a Km of 0.5 μM for the substrate UbAMC. (See, *e.g.*, Figures 40-42.) UbCHO was also demonstrated to be a specific inhibitor of BAP-1. (See, *e.g.*, Figure 43.)

Assays for ubiquitin hydrolase activity (*e.g.*, to assay BAP-1 activity) can also be performed as described in U.S. Patent No. 6,307,035 and Mayer and Wilkinson, *Biochemistry* 28:166(1989) using the glycine 76 ethyl ester of ubiquitin as a substrate. Peak areas can be integrated and normalized with respect to the ubiquitin standard.

Example 14: NP95 WT protein, ring finger mutants, siRNA and functional hit are antiproliferative.

The NP95 (G1-2635) functional hit (G1-2635) identified in the retroviral screen is in the sense orientation. (Figure 5). Expression of the functional hit in a tumor cell line, *e.g.*, A549 cells, or in untransformed cells, *e.g.*, HMEC or PrEc cells, was antiproliferative. (See, *e.g.*, Figures 6, and 44-45.) siRNA molecules derived from the NP-95 nucleic acid were shown to be antiproiferative in PrEc and HUVEC cells and H1299 cells. (See, *e.g.*, Figures 46-47, and 57.)

Using real time PCR analysis, NP95 mRNA expression was shown to be overexpressed in tumor tissue relative to normal tissue from the same patient. Increased